THE BACTERIOLOGY OF PLAGUE *

A REVIEW

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INTRODUCTION

Although there are apparently no data available to prove that the strains from the various animal species which are liable to natural pasteurellosis are themselves specifically distinct, it has become customary to give a specific name to each organism, corresponding to the animal from which it has been derived; thus, we have *Pasteurella suiseptica* from pigs; *Past. leptiseptica* from rabbits; *Past. aviseptica* from fowls; *Past. vituliseptica* from calves; *Past. boviseptica* from cattle; *Past. muriseptica* from mice and *Pasteurella ovisep-tica* from sheep. The condition produced by such organisms has been called haemorrhagic septicemia, and the organisms themselves are frequently referred to as the haemorrhagic septicemia group.

The organism producing pseudotuberculosis in the guinea pig (*Past. pseudotuberculosis*), and the organism producing plague in man (*Past. pestis*), resemble those producing haemorrhagic septicemia in so many respects, that they appear to be closely related.

The first member of the group was isolated by Kitt in 1878 from an epidemic disease affecting hogs and deer. Hueppe, in 1886, found that the organisms of fowl cholera, rabbit septicemia, haemorrhagic septicemia of cattle, and swine plague, resembled each other in certain cultural characters, and that the diseases caused by them were similar. He regarded the four organisms as varieties of the same species, and gave them the name *B. septicemiae haemorrhagicae.* Lignieres [1] (1900), defined the biology of these organisms more closely and, adopting the nomenclature proposed by Trevisan,

gave them the name Pasteurella, in commemoration of Pasteur's work on fowl cholera. Later investigations have established the fact that similar organisms occur throughout the whole animal kingdom, though Lignieres' belief in the extent of their aetiological significance is not shared by modern workers, and some of them have been considered secondary invaders to the virus actually responsible for the disease. Malassez and Vignal[2] (1883) were the first to describe the pseudotuberculosis in the guinea pig. Pfeiffer[3] (1890) recorded finding a bacillus in the disease and named it *B. pseudotuberculosis.*

Since we are primarily interested in plague as an important tropical disease, we will discuss here *Past. pestis,* mentioning the other organisms of the group only when their relations to *Past. pestis* justify it.

HISTORY

There are records describing a ravaging epidemic as early as the year 430 B. C., but the symptoms described in this epidemic do not conform with any of the known clinical entities of the present-day plague.

About the year 300 B. C., a physician who lived in the time of Trajan described a fatal malady characterized by high fever, delirium, pains, and the presence of buboes.

More complete records are available of other epidemics such as that which occurred during the reign of the emperor Justinian (542 A. D.), and that lasted for the greater part of half a century. The "Black Death" or Great Mortality (1347–50) was also a world-wide event. Prior to this epidemic, waves of infection coming into Europe were traced back directly or indirectly to Africa; but at this later date the outbreak occurred in China (1334), and gradually extended eastward and westward, invading Europe by way of the Crimea and the Black Sea. In less than ten years the disease had practically spread all over the known world. From this time on, the plague was established as an endemic disease in various points of Europe, and as a result of this great calamity, quarantine laws were enacted for the first time.

During the 15th and 16th centuries, plague epidemics occurred in England, Egypt, Germany, Holland, Italy and

Spain. Among these was that of Naples in 1656, which killed 300,000 persons in five months, and the Great Plague of London (1664–1666), which caused over 70,000 deaths in a population of 450,000. Then came what is generally known as the Period of Eastward Recession in which the plague definitely receded toward the Orient. Three notable exceptions in this period were the epidemic of Marseilles (1720), the epidemic of Messina (1743), and that of Moscow (1771).

In the beginning of the 19th century, the plague was limited to a few endemic centers in the countries bounding the eastern Mediterranean and the Caucasus. From these points occasional outbreaks spread to southern Russia, the shores of the Adriatic, and to the Danubian countries, but none of these invasions were considered very serious, and usually disappeared spontaneously.

In 1815 plague appeared at Noya in eastern Italy, but remained localized in a small district. In 1833 and thereafter, a number of epidemics raged in Egypt. It was at this time that the first serious attempt to study plague scientifically was made. Unfortunately, the work of the French Commission to Egypt was interrupted by the sudden disappearance of the infection from the country.

Plague finally left Europe in 1841; ten years later it disappeared from Armenia, and in 1845 from Syria and Egypt. Thus, the Old Levantine Plague vanished. The present pandemic has its origin, apparently, in the Chinese province of Yuman. It is well known that in the year 1860, and again in 1871, the disease was active in this region. From here the disease spread over a large portion of southern China, reaching the port of Pakhoi in the Tong King Gulf in 1882. It is said that plague was prevalent in this region for 15 years, and it may have passed from here to Canton, where it appeared early in 1894. Thence it was carried by steamer to Hong Kong in May of the same year. About this time the disease appeared in Bombay, and during 1896–1920 plague attained world-wide dissemination.

It was in the Hong Kong epidemic that the cause of plague, *Past. pestis,* was discovered. Two Japanese investigators, Kitasato and Aoyama, were sent by the Japanese government to study the disease. They arrived in Hong Kong on the 12th of June, and on the 14th, Aoyama performed an

autopsy. In the buboes, blood and spleen of this case Kitasato found a definite bacillus. He cultured the same organism from the blood of patients with plague, and inoculated mice, guinea pigs, rabbits and pigeons. With the exception of the pigeons, all the animals succumbed to the disease, and the same organism was isolated from their viscera. The French bacteriologist, Yersin, who was also in Hong Kong, made similar investigations at the same time, and isolated a bacillus similar to that discovered by Kitasato. It was also about this time that Roux and Yersin definitely suggested the relation of rat plague to human infection. (For more detailed studies of the discovery of *B. pestis* see Lagrange [4] and Severn [5].)

In the Indian epidemic, studies on the bacteriology of plague were carried out by Hankin and Leumann [6] (1897) and Childe [7] (1898) and by commissions appointed by the various European governments. The results of their work is published in a series of reports, among which are: The Austrian (Albrecht, Müller, Ghon and Poeck [8], 1898–1900), the German (Gaffky, Pfeiffer, Stricker and Dieudonné [9], 1899), the British (Trasser, Wright and Ruffer [10], 1899–1901), the Russian (Wyssokowitz and Zabolotny [11], 1897) and the Indian Plague Commission [12].

Later, in 1905, the Royal Society and Lister Institute published a long series of reports (1906–11) which did a great deal to further the knowledge of plague. Protective inoculation was carried out on a large scale by W. M. Haffkine in 1897. Plague serum was used by Yersin, Calmette and Borrel [13] in 1895.

The pathological anatomy of plague was thoroughly investigated by the Austrian Plague Commission in which H. Denck, 1904, was very prominent. Recent investigation has been concerned with its modes of transmission.

Very little was known in ancient times about plague in animals. There are records reporting that in the Black Death of Constantinople, mice died. Apparently, the black rat (*Mus rattus*) was unknown to Greeks and Romans, and came first to the shores of Europe in a Crusade ship from the East. The common rat (*M. norvegicus, M. decumanus*) was noted in Copenhagen in 1716. From the time of the Canton epidemic, plague in rats has been found in the

majority of epidemics all over the world. The mechanism of the transmission of plague to man was subject to controversy for many years. Ogata [14] (1897) suggested the flea as a possible vector. Simond [15] (1898) and Gauthier and Rayband [16] (1903) and later W. G. Linton [17] (1905) specially developed the flea theory. Linton's ideas were taken up and thoroughly investigated by workers of the Indian Plague Commission.

CLASSIFICATION

The members of the Pasteurella group have been considered by many as constituting a single species which has only suffered slight modifications as regards to virulence by prolonged existence in different hosts. Other investigators have considered the various types to be more distinct, and have recognized the necessity of raising the group to the status of a genus.

A classification based on morphological and cultural characteristics has been impossible. Such features only serve to establish the identity of the group. Although there are certain divergences, as for example, in the fermentation of carbohydrates, they do not serve as a basis, and do not coincide with the animal origin of the strains.

Serological classification has been attempted by a number of workers, employing agglutination, absorption and complement fixation, but none of these methods has given a satisfactory grouping. A classification by pathogenicity has been impossible, since cross-infection is possible to a notable degree.

As none of these methods of classification is reliable as a means of dividing the Pasteurellas into types, we must conform ourselves to the zoölogical classification, which arranges the organisms according to the animal source of the original strain. Such a classification must be recognized as purely arbitrary, but may lead to future work in demonstrating the existence of clearly marked varieties.

Pasteurella pestis and *Pasteurella pseudotuberculosis* stand apart from the remaining members of the group which caused hemorrhagic septicemia in animals, by their ability to grow on media containing bile salts, their inability to ferment saccharose, and the lack of production of indol and hydrogen sulphide.

MORPHOLOGY AND STAINING

All members of the group are small ovoid bacilli, having convex sides and rounded ends, but no characteristic arrangement; they may be found singly, in pairs or short chains, or in groups.

The most striking point is their pleomorphism, which is most marked with *Past. pestis*. *Past. pestis,* when examined from tissue smears, is seen as an oval rod with convex sides and rounded ends, measuring from 1.5 to 1.75 μ long and from 0.5 to 0.7 μ broad. They show bipolar staining and may be found singly, in pairs, or in groups. Forms like cocci, streptococci, streptothriciae, and even mould and yeast forms have been described under the name of involution forms.

Albrecht and Ghon [8] (1898) stated that 5 per cent glycerin agar and a medium containing 5 per cent of sugar, favored the appearance of polymorphous forms of plague bacillus. Hankin and Leumann [6] (1897) reported that the addition of 3 per cent sodium chloride to agar produces striking variety of bizarre forms which are of diagnostic value.

The Plague Research Commission in India reported that on rare occasions the plague bacillus, found in dead rats with acute plague, resembled small coco bacillus, and that the abscesses found in the spleen of rats suffering from chronic plague sometimes showed involution forms. These so-called involution forms are very important from the standpoint of diagnosis. The concensus of opinion is that they are not degeneration forms, since they yield normal cultures which on inoculation produce typical infections.

Kitasato [18] (1894) was the first to report that *Past. pestis* may form a true capsule. Rowland [19] (1914) confirmed the existence of a capsule in this organism and reported further that it is insoluble in water but soluble in weak alkali. It can be observed best when the organism is grown in 10 per cent inactivated horse serum broth, incubated at 36°C. *Past. aviseptica* may also show an indefinite capsule.

The organisms of this group are all non-motile with the exception of *Past. pseudotuberculosis,* which shows motility in broth cultures grown for 18 hours at 20°–22°C. This point is of importance in differentiating this organism from *Past. pestis* with which is may be easily confused.

All members of the group are Gram negative, and stain rather well with the ordinary aniline dyes. To obtain bipolar staining, preparations can be dried and then treated with alcohol or alcohol-ether mixture for 1 minute; the fluid is then poured off and the stain added. Dilute carbolfuchsin, carbolthionin, methylene blue, and Archibald's stain are the most commonly used, for staining the organism in the tissue, they can be fixed with corrosive sublimate solution, with alcohol, or with Orth's fluid.

CULTIVATION

Pasteurella pestis grows well on the ordinary laboratory media, the temperature range for its growth being from 4 to 43°C. with an optimum temperature of about 30°C. Growth takes place best in a neutral or weakly alkaline medium, showing a pH range of from 5°–8.2, with an optimum of 6 or 6.6.

Most authors stress the necessity of air for its growth; however, when cultivated in broth over which coconut oil or clarified butter is floated, the organism produces a characteristic stalactite growth suspended from the oily layer.

On the Agar Plate: Very small, round, glistering transparent, colorless finely granular, umbonate colonies developed after 24 hours incubation at 37°C. The surface of these colonies is very smooth or finely granular and an entire or delicately notched edge, differentiated by a raised center and a flat periphery.

On the Agar Stroke: A poor, slightly raised, translucent, greyish-yellow growth develops after 24 hours incubation at 37°C. The surface of the growth is usually wavy or like frosted glass with an irregular lobate edge.

In broth: There is moderate growth in 24 hours with little or no turbidity. A powdery or floccular deposit not disintegrating completely on shaking is formed, and on ageing it crawls up the sides of the test tube. A delicate surface pellicle often forms on potatoes. The growth is not satisfactory.

Slimy growth: There are certain factors which influence markedly the growth of the cultures producing slimy growth. Some authors attribute this slimy appearance of the growth to the capsule described by Rowland. It seems as if the tem-

perature of incubation, the reaction and moisture of the medium, and the growth in the animal body, have a decided influence in producing sliminess. Thus, Dieudonnée and Otto [20] (1927), relating their own experiences and that of other authors, report that non-slimy growths recover their character when passed through animal tissues and that cultures grown at 37°C. are more slimy than those grown at 25°C.

Autolysis occurs in old cultures of *B. pestis*, and is due to the breaking down of the protein by means of a proteolitic enzyme which is set free during the process of disintegration of the organisms after their death.

Dissociation: Gottschlich [21] in 1912 described slimy, undifferentiated colonies of *B. pestis* which were poorly agglutinated and were avirulent for rats, but subsequently reverted to the virulent type.

In cultures of *Pasteurella leptiseptica*, De Kruif [22] (1921–22–23) found two different types: Type D grew diffusely in broth, formed rather opaque, fluorescent colonies on serum agar, and was highly virulent for rabbits, while type G gave a granular deposit in broth, formed translucent bluish colonies with little fluorescence, and was completely avirulent. Type D gave rise to G variants, but G did not revert to D.

Variants have also been described for *Past. pseudotuberculosis* by Kakehi [23] and Zlatogoroff and Moghilewskaja [24]. It is quite difficult to differentiate between *B. pestis* and the other organisms of this group. This differentiation is most difficult in the case of *Past. pseudotuberculosis*. Probably the most useful distinction between the two organisms *Past. pestis* and *Past. pseudotuberculosis*, is that the latter is motile in broth cultures incubated at 22°C. for 18 hours, while *Past. pestis* is uniformly non-motile. Agglutination and precipitation may be of assistance in the differential diagnosis of these two organisms, specially if aided by absorption.

The comparative harmlessness of *Pasteurella pseudotuberculosis* for white rats, and its alkali production in litmus milk, may be also of value in differentiating it from *B. pestis*. McConkey [25] has laid stress on the inability of *Pasteurella pseudotuberculosis* to grow in bile salt media, but Topley and Wilson [26] report that this characteristic is by no means constant, and that by using a generous inoculum they have had no difficulty in obtaining growth.

The haemorrhagic septicemia group of organisms can be differentiated from *B. pestis* and *B. pseudotuberculosis* by their ability to ferment sucrose, their production of indol, and their negative methylene-red reaction. One must have in mind that sucrose may be fermented by certain strains of *Past. pseudotuberculosis.*

Filterable Forms: There is evidence suggesting the existence of a filterable form of this organism. E. Burnet [27] reports having cultured *B. pestis* from filtrates of suspensions prepared with the organs of infected mice and guinea pigs. According to his report, the filterable forms consist of granules derived from the lytic disintegration of the bacillary bodies. Hnadinoy and Ghalib [28], while working in antiplague bacteriophage, observed that some filtrates of plague cultures gave rise to secondary cultures of the organism.

BIOCHEMICAL PROPERTIES

Action on Carbohydrates: In practising fermentation reactions with this group of organisms, great care is necessary, since their growth in sugar peptone water media is very poor.

Pasteurella pestis ferments a number of carbohydrates with the production of acid, but no gas. After studying the reports of various authors, one comes to the conclusion that glucose, levulose, manitol, maltose and galactose are invariably fermented, whereas lactose, raffinose, saccharose, dulcitol and inulin usually undergo no change.

Some authors report that salicin is usually fermented, fermentation taking place within ten days. *Pasteurella pseudotuberculosis* ferments also glycerol, and in some instances produces acid in sucrose.

Most of the organisms of the haemorrhagic septicemia group ferment glucose, manite, sucrose and maltose producing acid. There are several reports in the literature indicating that a number of strains produce acid in lactose and to a less extent in salicin.

Action on Proteins: There is a general agreement that indol is not produced by *Past. pestis* and *Past. pseudotuberculosis* and that they both give a positive M. R. reaction, whereas *Past. aviseptica* forms indol and gives a negative M. R.

Past. pestis and *P. aviseptica* have no action on litmus milk while *P. pseudotuberculosis* turns it slightly alkaline. All members of the group reduce nitrates to nitrites, form ammonia and H_2S, and give a positive catalase test.

Autolysis: The process of maceration of the bacilli after death brings about a subsequent breaking down of the protein by means of a proteolitic enzyme which is set free. In a four-day-old broth culture tested by Rowland [29] no coagulable protein was present, but after 14 days there was a considerable amount.

Toxin Production: There is an amount of evidence throughout the literature to prove that the toxin of the plague bacillus is an endotoxin set free as a result of disintegration of the dead bacillary bodies. Up to the present it has been impossible to separate the constituent antigens of the organism in constituents of bacterial protein.

The products extracted from cultures of the plague bacillus give the reaction of a nucleoprotein and are precipitated by acid, which precipitate can be redissolved in dilute alkali.

Apparently, the immunizing substance is closely associated with the protein, and attempts to separate it have failed. According to the work of Rowland [28] one-tenth by weight of the whole bacillus is nucleoprotein. (For detailed studies of the toxin see section on Immunity.)

RESISTANCE

The members of this group possess feeble powers of resistance to adverse influences. They are very sensitive to the action of disinfectants and are easily killed by heat. Broth cultures are killed at 150°C. and by 0.5 per cent phenol within 15 minutes. On the other hand, they can survive at a temperature of 0°C. for forty days. They are quickly killed by drying, but may remain alive for a considerable time if they are in contact with substances such as mucus and proteins, that protect them from desiccation. In juice from tissues dried over a cover slip, *B. pestis* dies in 4 days (Kitasato 1894). *Past. pestis* does not thrive well in the presence of other bacteria; in the living tissues it is easily overgrown by other bacteria such as the pyogenic cocci, and in dead tissue it is soon overgrown by the putrefactive. Water is a comparatively inadequate medium for plague

bacillus to live in and multiply. In sterile, distilled water kept at a temperature of 20°C., the organism can live for from 30 to 60 days; if there are other bacteria in the water, its life is considerably shortened.

The viability of the plague organisms in the air is of importance if we consider that a form of the disease attacks the respiratory tract causing an infectious pneumonia. Teague and Barber [30] report that bacilli contained by the sputum droplets lose their vitality easily by desiccation, but this may be prevented by favorable atmospheric condition and the life of the organism considerably lengthened. When relative humidity is high and the temperature low, the organism may survive longer. Apparently, these factors influence the occurrence of pneumonic plague in the temperate ones, where cold and moist atmosphere is more prevalent than in tropical regions.

Formerly it was thought that plague could be transmitted through infected merchandise. Scores of investigators have proved that the danger of infected merchandise as a means of propagation is not due to direct contamination of the articles, but probably to the existence of infected rodents in the goods.

Ostertag [31] found that organisms in the blood of animals dying of hemorrhagic septicemia may remain virulent in the dried state for about 3 weeks. Blood which was allowed to putrefy in a glass tube may remain virulent for 100 days.

SEROLOGICAL VARIETIES

A serological classification of the Pasteurella group has been attempted by a number of workers, but so far no satisfactory grouping of the various types has been accomplished.

Chamberland and Jouan [32] found that the strains they were working with lose their agglutinability with great ease. The observations of a number of workers show that serum prepared against any one member of the hemorrhagic septicemia group will agglutinate the others to titre. Direct agglutination, therefore, fails to identify the animal source of the strain.

With regards to *Pasteurella muriseptica,* Topley and

Wilson report two distinct types distinguishable by direct agglutination and by absorption.

The relation between *B. pestis* and *B. pseudotuberculosis* is very close and it is often impossible to distinguish them by direct agglutination. Ackewright [33] has shown that agar cultures of *Pasteurella pseudotuberculosis* incubated at 22°C. for 18 hours contain a heat-liable H-antigen and a heat-stable O-antigen. The heat-liable antigen is destroyed by boiling a half hour; the heat stable is not destroyed by boiling. Schutze [34] (1928) has confirmed this work and finds that the H-antigen is common to all *Past. pseudotuberculosis* strains, whereas there are several types of O-antigen, one of which seems to be related to the Salmonella group of organisms.

As seen by the above, the Pasteurellas cannot be divided as yet into clearly marked serological varieties.

IMMUNITY

Agglutinins: Agglutinins are frequently absent from the serum of plague patients, and when they occur their titre is quite low. The agglutination test is not used in this as in other conditions, because agglutinins are frequently absent from the serum of the plague patient. There is also a marked tendency to spontaneous agglutination on the part of the plague bacilli.

Some authors (Stepanoff and Grigorieff [35], and Markl [36]) have used acid agglutination with the plague bacillus. Markl found the optimum reaction to occur at pH 3.8–4.1, and believes that the test may be useful for the purpose of identification. In our personal experience [37] in the second epidemic of Bubonic plague in Puerto Rico (1921), we had no difficulty in immunizing rabbits against different strains of *B. pestis*. Agglutinations were obtained with the homologous as well as the heterologous strains in dilutions from 1–10 to 1–300; most of the rabbit serum, however, gave clear-cut results to dilutions of 1 to 150 only. Of several human sera examined from convalescent cases, 5 showed agglutinins from 1 to 80 to 1 to 120, 2 showed titres of 1 to 20 and 1 to 40 only, and 8 showed no agglutinins. It must be taken into consideration that these cases had been treated with large quantities of antiplague serum.

Alexin Fixation: Alexin fixation has been demonstrated by several authors. Matsuda [38] (1910) employed alexin fixation in comparing several organisms of the haemorrhagic septicemia group. Bukofzer [39] (1922) reported that *P. aviseptica* fixed complement in the presence of aviseptica sera. Roderick [40] (1933) tried to classify the Pasteurella by alexin fixation and Lal [41] (1927) compared the complement fixation properties of five animal types of Pasteurella and found cross-fixation between all types except leptiseptica.

During the second epidemic of plague in Puerto Rico we were able to demonstrate alexin fixation in four cases recovered from the disease. All these cases had been treated with large doses of plague antitoxin; however, in other cases that were similarly treated, no alexins could be demonstrated. Rabbits immunized with *B. pestis* strains from different sources showed alexins in their blood serums. Rabbits' immune sera gave complete fixation with the corresponding antigen in dilutions of 0.01 cc.

Precipitins: In 1897 Kraus [42] found that plague-immune serum caused specific precipitate when mixed with extracts of plague bacilli. The precipitin test is said to be more delicate than the agglutination or complement fixation test. The antigen is prepared by suspending an agar culture in distilled water, adding 0.5 per cent phenol, shaking for 24 hours and centrifuging until clear. Zlatogoroff [43] reports that the pseudotuberculosis antigens fail to react with plague serum. Warner [44] observed a group reaction between these two organisms. The thermoprecipitin reaction has been used in the diagnosis of putrid plague-infected rats (Piras [45]). The test consists of adding to specific serum in a test tube the filtrate of a boiled suspension of the organs of the suspected animal. If the corresponding bacterial antigen is present a precipitation ring quickly forms at the surface of contact. Pulgher [46] (1922) prepares the extract by adding the suspected liver and spleen to an equal volume of saline, then boiling and then filtering. The reaction is read after 20 minutes incubation at 37°C. and again after 1 hour at room temperature.

Toxin: No true toxin is formed (Hadley [47]). Old broth cultures are very toxic, suggesting that endotoxins are liberated by autolyses of the bacilli. Rowland [29] (1910)

working with *B. pestis* observed a toxic nucleoprotein by treating the bacillary paste scraped from surface agar growths with anhydrous sodium sulphate. According to the same author, the virulence of the culture is not related to its toxicity. The endotoxin, when kept in fluid, is not very stable if stored at room temperature. At 37°C. it suffers a considerable loss in toxicity. When kept at a temperature below 3°C. the toxicity is well maintained. Toxin heated from 50° to 70°C. for 1 hour markedly loses its power. The nature of the toxin is not yet understood.

Rowland reports that toluol or chloroform are suitable preservatives for the toxin. Thymol was found to lessen its toxicity and vaccination value, and phenol was also harmful.

Guinea pigs and rabbits are very resistant to plague toxin, whereas mice and rats are very susceptible. Mice are usually killed in about 18 hours, and rats in from 24 to 48 hours. The lethal dose varies with the strength of the preparation. Rowland found that his nucleoprotein killed rats in a dose of 0.1 mg.

Markl and also Rowland were able to immunize animals by administering sublethal doses.

Natural Immunity: Natural immunity is apparently very rare. Birds, with the exception of the sparrows, are completely resistant. Dogs, cats, pigs, cattle, sheep, goats and horses can be infected with difficulty.

Active Immunity: A single attack of plague usually confers lasting immunity. Second attacks rarely occur. Many experiments have been reported on the immunization of experimental animals. It appears from them that plague vaccines confer a definite immunity upon the rat and upon the guinea pig, that the rat is more easily rendered immune than the guinea pig, and that the degree of protection depends on the thoroughness of the immunization and the severity of the testing procedure.

In man, vaccination has been used extensively. Kolle [48] prepared a vaccine by emulsifying surface agar cultures of the bacillus in normal saline. After shaking, the emulsion was heated at 70°C. Lustig and Galeotti [49] employed a toxic precipitate obtained by treating the bacilli with sodium hydroxide and acetic acid. The substance was dried and dissolved in sodium bicarbonate. We have already men-

tioned Rowland's sodium sulphate method. Besredka [50] made a vaccine composed of heatkilled cultures impregnated with anti-plague serum.

Strong succeeded in vaccinating individuals with attenuated living cultures. The most popular vaccine seems to be that of Haffkine [51] which is prepared from a 2 to 6 weeks culture of *Past. pestis* at 25 or 30°C. in goats digest broth, and killed by heat for 1 hour at 65°C. and to which .5 per cent phenol is added to maintain sterility. The dose of the vaccine for an adult is from 3 to 3.5 cc. A second dose should be given 7 or 10 days after the first.

The protection of the vaccine is not absolute, but it sensibly diminished the incidence of plague attacks on the inoculated population. The protection conferred by inoculation lasts several weeks, possibly for months.

From the work of Markl [52] (1903) and Rowland [53] (1912) it seems as if active immunity in plague depends principally on the efficiency of the phagocitic and lytic process of the organism.

Flu [54] (1929) reports immunizing rats with plague cultures that were previously lyzed by anti-plague bacteriophage. Compton [55] (1930) has confirmed these experiments and advocates the use of the increasing doses, for 1 or 2 doses are not only insufficient to give protection, but may often lead to an increased susceptibility.

Passive Immunity: Anti-plague serum has been used both for prophylactic and for curative purposes. It is claimed that 10 cc. of the serum can be administered as a prophylactic dose and that a state of passive immunity is conferred on the individual that lasts for 10 days. Much larger doses are used for curative purposes. Lavandero [56] administered 250 cc. at a time, intravenously. This dose was frequently repeated a second and a third time during the 24 hours.

Early authors report definite curative properties with the serum, using experimental animals. According to McCoy and Chapin [57] (1920), in actual practice no serum has given unequivocal results. Lavandero [56] (1923) reports that the serum is of value if used early enough and in sufficient quantities.

In the experimental animal, administration of serum promotes phagocytosis and lysis of the organisms. Sensitized

bacilli, when inoculated into the peritoneal cavity of the rat are readily phagocytosed.

Brooks [58] (1914) obtained an opsonic response by inoculating white rats by injecting them with the soluble bacterial portion. Most authors have failed to demonstrate a bactericidal element in immune serum, although it contains specific amboceptors.

Bacteriophage: D'Herelle [59] has reported the isolation of a bacteriophage which is active against *Past. pestis,* and has advocated its use in the treatment of plague. This form of treatment has been tried in India, but has not so far proved its efficacy (Mackie [60] (1928)).

PATHOGENICITY

The haemorrhagic septicemia group of organisms is pathogenic for a large number of animals and birds but not for man. *Pasteurella pestis* and *Pasteurella pseudotuberculosis* cause disease in rodents. *Past. pestis* is pathogenic for man.

The Rat: Rats have for many years attracted attention with regard to the role they play in the propagation of bubonic plague. There are several species of rats: *Mus rattus,* the ordinary rat, almost uniform in color, tail and loins very dark gray, tail longer than the body (including the head), smooth palate, slender build, less fierce, and domestic in habits; *Mus alexandrinus,* similar to the Rattus species but having a white belly; *Rattus norvegicus* or *Mus decumanus,* a large, robust, fierce rodent, which lives in underground hollows. Its ears are small and opaque, and its tail shorter than the body and head taken together. Measures about 52 cm. long, and the central part of the loins are commonly darker than the sides; the palate is very rough. When a rat is infected, it usually sits quietly hunched up, or staggers around lazily. The rat shows at post-mortem examination a marked congestion of the subcutaneous tissue with haemorrhagic infiltration. This was a very important finding in the epidemic of Puerto Rico, and occurred in 90 per cent of the rats examined (Morales Otero [61] (1927)). The bubo is a reliable sign of plague in the rat. The Plague Research Commission in India regarded a typical primary bubo as the most important sign of plague, 75 per cent of the buboes occurring on the neck. This was not the case in Puerto

Rico, where we found the haemorrhagic infiltration to be the most common lesion. Haemorrhagic and swollen buboes were commonly found by us in the inguinal, axillary and cervical regions. Those located in the inguinal region predominated. Messenteric and pelvic buboes were very rare. The liver is usually enlarged and friable, dark red in color or may show small yellowish granules. Swollen, dark, congested livers showing necrotic areas were found in 73 per cent of all positive rats examined during the epidemic in Puerto Rico. The spleen is usually enlarged and may be covered by small surface granules. Patches of broncheo-pneumonia with pleural adhesions, and pleuresy with effusion, were not uncommonly found.

Rats without Lesions: In 1910 McCoy [62] observed rats which showed no gross lesions of plague and were positive to guinea-pig inoculation. We also found [61] (1921) rats that showed no macroscopic lesions, but marked emaciation and intense anemia, which was recognized by the pale appearance of the tissues which were practically loaded with virulent plague bacilli.

Williams and Kemmerer [63] (1923) in New Orleans, and Bordas, Dubief and Tanon [64] (1922) have reported similar observations.

Rats with Chronic Lesions: Chronic plague lesions in the rat have been described by Kolle and Martini [65] (1902), and by Albrecht, Ghon and Hata [66] (1904). The lesions found by these workers were cheesy, bronchial glands, solid induration of the lungs, and encapsulated foci in the submaxillary glands. The Plague Research Commission in India reports naturally occurring resolving plague in rats, recognized by the presence of purulent or caseous foci in the peripheral or messenteric glands, and very particularly in the spleen.

The Guinea Pig: The most common method of experimental infection of the guinea pig consists in rubbing the infected material over the skin of the previously shaved abdomen. Usually thirty-six hours after inoculation, the animal begins to show symptoms. It appears indifferent and indolent, gradually its head falls between its front legs and breathing becomes rapid, temperature high, its hair bristles, emaciation occurs rapidly and from the fourth to the fifth day the animal dies. On autopsy, a haemorrhagic exudate

is usually found at the site of inoculation. The lymphatic glands corresponding to the region of inoculation are all swollen and surrounded by tremendous infiltration. The spleen in most cases is enlarged, deeply congested and full of small yellowish nodules, like miliary abscesses. The liver is large and congested, the gall bladder is usually dilated and distended with fluid and the kidney and suprarenal capsules are enlarged and congested. A very extensive bilateral bronchopneumonia commonly occurs. The viscera contain enormous quantities of plague bacilli that can be easily demonstrated by culture or by direct smear.

Swellengrebel and Otten [67] (1914) described a variety of plague in the experimental rats and guinea pigs which they termed mitigated plague. In 1921, during the epidemic that visited Puerto Rico we found (Morales Otero, 1927) that not all of the guinea pigs inoculated with plague material died of the acute form of the disease. Some resisted the infection and when autopsied three weeks after inoculation, lesions caused by plague with the presence of plague bacilli were found. This shows the importance of autopsying every pig inoculated with suspicious material even if it survives inoculation.

The Tarbagan: The tarbagan (*Arctomys bobac*) is a rodent about 37 centimeters long weighing from 6 to 8 kilos. It lives in burrows below the surface of the ground and is indigenous to the immense mountain regions extending over central Asia. The animal hibernates from October until March or April. It is very valuable for the high quality of its furs, and also as a source of food. The occurrence of a mortiferous, plague-like disease in this animal, which is transmissible to man, led to the study of this condition. It was found that human disease was always preceded by tarbagan epizootics. Further epidemiological studies proved that the Manchurian epidemic (1910) had its origin in the tarbagans of that region, and was spread throughout the country, as infected hunters returned to their homes.

The infected animal looks weary and exhausted. A condition of ischemia is most frequently found in the paws, and as a rule there is an inflammation in the glands of the axillary region. Other anatomical lesions are essentially the same as those found in the rat and guinea-pig. Dujardin,

Beaumetz and Mosny [68] inoculated two hibernating animals that survived from 61 to 115 days, while a non-hibernating control died of plague shortly after inoculation. Apparently, the physiological phenomena associatel with hibernation lowers the virulence of the organisms and the animal is able to survive throughout the winter, thus forming a natural reservoir of plague throughout the year. Strong [69] inoculated several tarbagans by different methods and reports some dying of acute plague, while in others he found subacute lesions.

The Ground Squirrel: In western United States infection has extended to another rodent (McCoy [70] (1910)), the California ground squirrel, which is allied taxonomically to the Asiatic marmot. Investigations of human cases occurring in Contra-Costa county in 1903–1904 showed that they presumably contracted their infection from ground squirrels, but it was not until August 1908 that it was definitely proved, bacteriologically, that plague infection existed among ground squirrels. Reports from reliable sources indicate that ground squirrels in the East Bay countries died of some epizootic previous to 1900 and it is possible that the disease affecting these rodents at that time was plague.

The ground squirrel (*Citellus beecheyi*) is not as large as the tarbagan. Its hair is of a yellowish color showing dorsally a number of dark longitudinal stripes. The tail, which is more than two-thirds the length of its body, is dark above and yellow below and is very well haired. The animal is terrestial in habits, dwells in underground burrows and is classed as a hibernating rodent, although its winter sleep is not as profound as that of the Asiatic marmots.

Since the presence of plague in the ground squirrel was proved, as much information as could be obtained on the life history and habits of rodents was disseminated in the infested areas in order to keep the residents on guard, and an intensive campaign to eradicate this animal was started. As a result, squirrel infection has ceased to be a serious problem in California; however, isolated plague-stricken squirrels now and then have been encountered.

The Spermophile: Recent observations seem to indicate that the endemic plague exists among another rodent, the spermophile.

Since 1910 it was observed that the occasional contact of man with the spermophile resulted in plague. This led to the study of this rodent, and in 1912 it was found (Bieliavsky and Rieshentnikoff [71]) that spontaneous plague infection occurred in a large number of these animals. Zabolotny [72] (1923) and others studied the question more in detail. Apparently, some of these animals resist plague infection better than others. The infected animals which have resisted infection hibernate each year. When the period of hibernation is over, the new generation is infected, and the epizootic reoccurs. Usually, epizootics occur during the months of May and June, *i. e.*, the period in which the new generation begins to take care of itself. Plague reaches man from these animals either by direct contact or by the agency of the fleas. The flea may also infect field mice and the mice disseminate the infection.

The Gerbile (Tatera Lobengulae): The gerbile has been found to act as a reservoir of plague in some districts of Cape Province in South Africa. The infection is transmitted to man through the agency of field mice (*Mus concha*) which are common visitors of the gebrile's den, and whose fleas are known to bite man readily in the absence of the proper host.

Man: Man is very susceptible to infection by *Pasteurella pestis.* When this organism infects man, a disease is produced which is characterized by extremely tender glandular enlargements, a dazed condition, high fever and prostration, or may appear as a fatal pneumonia. Either the pneumonic or bubonic types may become septicemic, or this form may exist from the start.

BUBONIC PLAGUE

After a period of incubation that may vary from 3 to 7 days, the symptoms may come on abruptly, or follow a prodromal stage with giddiness, pains in the back and limbs and general malaise. The patient has a pale, drawn, anxious expression, the speech becomes thick and difficult, there is stupid mental state, and a tendency to wander aimlessly about. Delirium is common, the fever rises rapidly and is often associated with shivering. The face becomes flushed, the conjunctiva markedly injected, the pupils dilated and the eyes staring. The pulse is rapid and cardiac weakness is

often marked. The urinary secretion is markedly diminished and shows large quantities of albumen and casts. On the whole, the disease is characterized by its toxicity on the nervous system, on the heart and on the endothelial lining of the capillaries.

On the third or fourth day, an extremely painful bubo develops or there may be multiple buboes. The buboes are characterized by the marked oedema of the periglandular tissues. As the case progresses, the anxious expression gives way to mental apathy. The control of speech is lost and the patient sinks into a typhoid state. The buboes may separate or may undergo slow resolution. Pulmonary congestion with dyspnoea, accelerated respiration and cough, are not infrequent, and at times broncho-pneumonia may develop. There is usually a marked leukocytosis with polimorphonucleosis.

PNEUMONIC PLAGUE

When the course of the disease is predominantly and primarily pulmonary, it is termed pneumonic plague. The onset is sudden with a marked rise in temperature, marked physical exhaustion and clouding of the consciousness. There is often dyspnoea and rapid shallow respiration, accompanied by cough and blood stained or sanguineous expectoration. The disease is transmitted from man to man by the droplets of sputum expelled in coughing.

The course of the disease rarely extends beyond the 4th day, ending invariably with death.

SEPTICEMIC PLAGUE

There are cases of plague which present no other symptoms than those of septicemia. Blood cultures are to be depended upon in diagnosis, and even these may be negative. The disease is usually fatal, patients dying within 48 hours.

EPIDEMIOLOGY

Plague is primarily a disease of rodents and secondarily of man. This fact was firmly established by the work of the Indian Plague Commission which discovered that plague may be transmitted from rat to rat and rat to man through the agency of the flea. It is now well known that the rat epi-

zootics preceding plague, are true plague. Rats are great travelers, and have carried plague to all quarters of the globe.

TRANSMISSION

Bubonic plague, which is the most prevalent form, is usually an insect-borne disease. In pneumonic plague, contagion takes place by direct or indirect contact with patients or affected rodents. Ogata[73] (1897) was able to cultivate *B. pestis* taken from the bodies of fleas on rats that had died of plague. Simond, in the same year, isolated the organism from the digestive tract of these animals. He also carried a series of experiments which proved without doubt that the fleas were responsible for the contagion. The English Plague Commission confirmed these findings, and further proved that a female plague rat could nurse its young without infecting them, provided that fleas were excluded. They also demonstrated that the fleas from an infected rat when separated from their host, and compelled to bite guinea pigs, reproduced the disease in these animals.

As a rule, acute plague in the rat is a septicemia and, consequently, any blood-sucking parasite will be inevitably infected. The plague organism multiplies freely in the alimentary canal of the flea and may be found abundantly in its feces. The deposition of infected feces in the small wound made by the insect while feeding makes infection possible in the human being.

The growth of plague bacillus in the proventriculus of the infected flea usually impairs the function of this organ, and permits the back flow of the infected material into the wound during the act of sucking, thus creating ideal conditions for infection.

Other parasites besides the flea have been incriminated as possible vectors of the malady. It is generally admitted that the bedbug, after feeding on contaminated blood, may remain infective for 4 or 5 days and yet may carry the disease to a new host.

Flies may convey the plague bacilli in their legs, wings or gastrointestinal canal. *B. pestis* has been demonstrated in the bodies of *Pedicule capitis* taken from the hairs of persons dying from plague. Some authors are of the opinion

that mosquitoes, under favorable conditions, may transmit plague.

Although these various observations do not offer definite scientific proof of the role played by these insects in the transmission of the disease, they offer a number of important possibilities worthy of further consideration.

The brown rat is more prolific than the others and is limited only by the food supply and opportunities to nest; it reproduces from two to five times a year, bearing usually six to nine young, although it sometimes may have as many as twenty-two or twenty-three offspring. They breed more rapidly in temperate and tropical climates.

The average life of a rat is usually about 2 years. They have great migratory habits. Seasonal movements of rats from houses and barns to open fields take place in the Spring when green and succulent plant food is ready for them. In England a general movement of rats inland from the coast occurs every October. This is known to be due to the close of the herring season. During the fishing, the rodents swarm to the coast, attracted by the offal left in cleaning the herring, and when this food fails, the animals troop back to the farms and villages. Rats board vessels readily, when they are docked. Sometimes they are carried on board in the cargo. It is through this sea-going tendency that the rat has become cosmopolitan.

The Flea: Since plague is an insect-born disease, and the flea is considered as the chief transmitting agent, the flea index expressed in the terms of flea per rat is considered perhaps the most important factor in epidemiology. It is probable that bubonic plague will not spread wherever the flea index is less than one.

The relative importance of the various flea species in the transmission of plague is a question which up to the present has not been definitely settled. It is thought, however, that the transmitting capacity of *Xenosphila cheopis* is superior to the others. Under laboratory conditions, infection has been carried by *Xenosphila cheopis, Xenosphila brasiliensis, Xenosphila astia, Pulex irritants* and *Ceratophyllus fasciatus;* also, by *Ctenocephalus canis* or *felis,* the dog and cat fleas.

Carrión [74] in a three-year survey (1931) made in San Juan, found five different species of fleas in the rats examined,

but *Xenosphila cheopis* represented 98.5 per cent of the total catch. The flea index was highest at the docks (14 per rat) and in the commercial section, (16 per rat).

Bubonic plague has occurred in nearly all parts of the world, but is confined chiefly to warmer latitudes (Robertson [75] 1923). Temperatures above 50°F. combined with a certain degree of atmospheric moisture seem to be the most favorable for its development and spread. According to White [76] (1918–1919), humidity is the most important factor in the spread of plague in India.

The relative absence of bubonic plague in cold regions has been ascribed to the deleterious influence of low temperature upon the process of flea breeding. Careful studies have revealed that egg laying and development from the egg to the adult stages are more active when the weather is wet and moderately warm, and least active when the relative humidity is low and the temperature is either too hot or too cold.

Pneumonic plague is usually considered a disease of temperate climates. *Bacillus pestis* is known to adapt itself to moderately low temperatures. The spread of infection is favored by the prevalence of a low saturation deficiency of the atmosphere, a condition which is propitious to the viability of the plague organism. Primary plague pneumonia is usually encountered in cold regions where the people are more predisposed to certain bronchial disturbances.

DIAGNOSIS

In Animals: The diagnosis is usually made by microscopic examination, culture and animal inoculation. The infected material is rubbed over the previously shaved abdomen of a guinea pig and the guinea pig observed and examined for typical lesion (see "Pathogenicity").

In Man: A high fever with marked prostration, severe nephritis and the presence of glandular enlargement should be suspected. The disease usually shows a marked leukocytosis with polimorphonucleosis.

The bubo should be aspirated and the fluid examined microscopically, by culture and by animal inoculation. In some cases of plague, the organism has been demonstrated by culture in the blood of patients suffering from the disease.

In cases where the diagnosis is doubtful, the bubo may be excised and injected into guinea pigs (Uriarte [77], 1925). In the early stages of the disease, a small vesicle may be found corresponding to the site of inoculation. Microscopic inoculation of the contents of the vesicle usually reveals the presence of plague bacilli. In pneumonic plague, large numbers of plague bacilli may be found in the sputum.

After death, the best material for investigation is the bubo and spleen. If the cadaver is putrid, the organs can be rubbed on the shaven abdomen of a guinea pig; microscopical and cultural tests can be also resorted to.

PREVENTION

The first essential for the suppression of any epidemic is the knowledge of the epidemiology of the disease in the particular community where it occurs. Proper authority is necessary in order to enforce all emergency measures. Success can only be accomplished when enough material resources are available. Epidemic campaigns against plague are usually expensive. A competent organization with laboratory and hospital facilities is necessary and a campaign of public education should be carried on at the same time that the disease is being attacked.

Importation of Plague: Plague infection is carried overseas in vessels. Maritime quarantine, therefore, is very useful in keeping out plague. In the United States, the Federal authorities keep careful records of the distribution of plague over the world, and vessels coming from infected regions are subject to precautions.

Measures are directed almost entirely against the rat. All vessels trading with plague-infected ports should be carefully fumigated. The egress and ingress of rats should be avoided. In cases where plague cases have occurred on board, the vessel is detained in quarantine, the sick are removed and isolated and all vermin are destroyed. When pneumonic plague has occurred, all the crew and passengers that have been exposed to infection are detained for seven days *. After plague has appeared in a community, all cases of the disease must be sought for and early diagnosis confirmed; all deaths from no matter what cause must be investigated and the body examined by an expert before

burial is permitted; a bacteriological laboratory is absolutely necessary. Cases of the disease should be rigorously isolated and the usual disinfection of urine and feces should be exercised. The room should be thoroughly disinfected to exclude all parasites. In cases of pneumonic plague, the sputum must be carefully handled and disinfected, and persons entering the room should be provided with masks that will protect them from inhaling infected material.

The eradication of plague would mean the extermination of rodents, which is a biological impossibility, for killing of large numbers give the survivors an easier living; so we must be satisfied with suppression and control. Several measures such as rat-proof buildings, traps, poisons, shooting, fumigation, bacterial viruses, keeping food from rats and natural enemies of the rat, etc., are used successfully as repressive and destructive methods.

Rat-proof buildings is a measure of first importance in excluding rats from a community. It eliminates all conditions that will favor in any way the permanence of rodents at a place for any length of time.

Anti-rat laws * and regulations have been enacted in several communities. These provide the rules to be followed in the construction of floors, walls, roofs and foundations of buildings of various types. Adequate methods for the storage of merchandise, food articles, proper disposal of garbage, etc. Rat-proofing should exclude all accessible food from the rat. Scarcity of food helps all other suppressive measures. Well-fed rats grow quickly, breed often and have large litters. The rat has its natural enemies such as owls, snakes, hawks, foxes, skunks, cats, ferrets, coyotes, minks, weasles and dogs. The persistent killing of these animals in some communities has been an important factor in the increase of the rats.

Among the several methods devised for the direct destruction of rats, trapping is probably one of the most important. Experienced trappers use spring, cage, guillotine, barrel and pit traps. It requires ingenuity to trap rats successfully—they are wary and avoid the smell of man. Cheese, bacon, grain, meat, fish heads, vegetables or bread may be used as baits. The Plague Prevention Service at San Juan, P. R.

* For details, see "Quarantine Laws and Regulations of the United States".

(Carrión [78] (1931)), has used coconut meat as bait very successfully for several years. It is very attractive food for the species and its cleanliness makes it especially desirable. Rats may be caught by spreading a mixture of resin and petroleum oil which has been boiled down to the consistency of glue on boards placed along runways.

Poisons are of service in granaries, stables, wharves, garbage dumps, etc. They are objectionable in dwellings, owing to the odor of dead rats, and most of them are dangerous to children as well as to domestic animals. The principal poisons used for rats are barium carbonate, arsenic and phosphorus.

Barium carbonate is usually made into a dough with four parts of flour or corn meal and one part of barium. Arsenic is popular and is used by mixing twelve parts of corn meal, one part of white arsenic (arsenous acid) and white of egg. Kitano [79] uses phosphorus, making it into bread which is cut into pieces containing 0.025 gm. of phosphorus per piece.

Poisons reduce rats, but do not eliminate them. They are of great value, however, in ridding large rat-proof structures of existing rats.

Fumigation is an efficient, radical method of rat destruction. Its use is limited to certain structures such as warehouses, ships [80] and sewers. Fumigation is accomplished by saturating the atmosphere of a tightly closed space with highly toxic gases or vapors. The substances mostly used are sulphur dioxide [81], carbon disulphide, hydrocyanic gas and carbon monoxide.

Bacterial Rat Viruses: In 1893 Danysz [82] reported a great mortality among the rats at Seine-et-Maine, caused by a coccobacillus. The organism described by Danysz seems to be closely related to the *Salmonella enteritidis* of Gartner. When inoculated into rats by feeding, it produces an epizootic among these animals. These experimental epizootics offer practically the same inconveniences as poisoning. Rosenau [83] reports that rats are notoriously resistant to bacterial infection; even plague fails to markedly diminish their prevalence. The claim that these rat viruses are nonpathogenic for man needs revision in view of the instances of sickness and death among human beings reported by some authors. Under natural conditions these rat viruses lost their virulence and notoriously failed in their action.

Rat surveys are of great value to determine the absence of rodent plague, especially at seaports.

According to Rosenau [83], the number of rats examined in these surveys should be, at least, 1,000 for every 10,000 of human population.

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